JoS JOURNAL OF STROKE

Journal of Stroke 2022;24(3):421-424
https://doi.org/10.5853/jos.2022.00486

Letter to the Editor

Neutrophil–Lymphocyte and Platelet–Lymphocyte Ratios Are Associated with Recurrent Ischemic Stroke in Patients with Embolic Stroke of Undetermined Source

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Dear Sir:

Many researchers have hypothesized that occult atrial fibrillation (AF) could be an etiological factor for embolic stroke of undetermined source (ESUS), since AF is often diagnosed after extended monitoring.[1] However, studies investigating oral anticoagulant effects in patients with ESUS did not report any benefit.[2,3] Thus, there is an increasing interest in alternative etiologies, including left ventricular dysfunction, atherosclerotic disease, and atrial cardiopathy without AF.

Systemic inflammation is crucial in atherosclerosis and contributes to coronary artery disease and ischemic stroke. The Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) and Colchicine Cardiovascular Outcomes Trial (COLCOT) showed reduction in cardiovascular events, including stroke, after canakinumab administration and daily low-dose colchicine, respectively.[4,5] Thus, attenuating subclinical inflammation could reduce cardiovascular events independent of lipid levels, possibly by increasing plaque stability and reducing progression.[6] A pro-inflammatory state associated with endothelial injury could predispose patients to thrombus and embolus formation. To the best of our knowledge, this hypothesis has not been tested in patients with ESUS.

The neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) are hematological markers of inflammation, and their correlation with venous thromboembolism, pulmonary embolism, and cardiac thrombus formation has been evaluated.[7,8] We aimed to examine the correlation between NLR and PLR and newly diagnosed AF and recurrent ischemic stroke in patients with ESUS.

We retrospectively evaluated 185 consecutive patients with ESUS admitted to a stroke unit at a tertiary hospital between 2014 and 2017. Ethics approval was obtained from the local institutional review board. Written informed consent by the patients was waived due to a retrospective nature of our study. ESUS was diagnosed according to consensus criteria: non-lacunar ischemic stroke, absence of atherosclerosis causing ≥50% luminal stenosis in the extracranial or intracranial arteries, left ventricular ejection fraction ≥30%, and non-identifiable cardioembolic source.[9] All patients underwent neuroimaging and vascular studies, 24-hour inpatient telemetry, and transthoracic echocardiography (TTE). Routine blood tests on admission included a full blood count (FBC), coagulation profile, creatinine and electrolyte levels, lipid profile, and glycated hemoglobin levels. FBC was performed using the automatic Sysmex XN-series Hematology Analyzer (Sysmex, Kobe,

Japan) with random sampling, and the results were verified by a hematologist. NLR and PLR were calculated from FBC at admission by dividing the neutrophil and platelet counts, respectively, by the lymphocyte count. Patients were followed-up for newly diagnosed AF and recurrent stroke, and prolonged cardiac monitoring with an implantable loop recorder (ILR) was offered at the clinician's discretion. In patients who declined ILR, AF development was assessed through clinical examination and electrocardiography during follow-up.

The independent t-test and chi-square test were used to analyze continuous and categorical variables, respectively. Binary logistic regression was performed to determine the association between AF and recurrent stroke. A multivariable logistic regression model adjusted for age, sex, hypertension, diabetes, AF, and left atrial volume index (LAVI) was used. NLR and PLR were analyzed in separate models to avoid collinearity. Receiver operating characteristic (ROC) curves were used to identify the best cutoff values of NLR and PLR to predict new-onset AF, recurrent stroke, and composite events of AF or stroke. Based on these cutoff values, we identified a high-risk population with high LAVI (≥35 mL/m^2) and high NLR or PLR.[10] This composite marker was assessed in relation to the same endpoints, as previously described. All statistical tests were performed using IBM SPSS version 26 (IBM Corp., Armonk, NY, USA), and statistically significance was set at P<0.05.

The mean age was 63.0±12.3 years with a median follow-up period of 2.1 years (interquartile range, 1.4 to 2.8); most patients were male (70.7%) and Chinese (69.2%). Seventy patients received an ILR (Medtronic Reveal LINQ, Medtronic Inc., Minneapolis, MN, USA). During follow-up, AF was newly diagnosed in 14 (7.6%) patients, while 19 (10.2%) developed recurrent stroke. Anticoagulation therapy was initiated in all patients with newly diagnosed AF; none experienced recurrent stroke during follow-up. There were no significant differences in demographics, comorbidities, laboratory findings, or major echocardiographic left ventricular findings between the AF and non-AF groups (Supplementary Table 1).

Both NLR and PLR were significantly associated with recurrent stroke (P<0.001 and P=0.011, respectively), which remained significant (P<0.001 for both NLR and PLR) after adjusting for comorbidities, AF, and LAVI (Table 1). The association with newly diagnosed AF was weaker for NLR (P=0.041) and absent for PLR (P=0.243). In the ROC analysis, the models showed a correlation with newly diagnosed AF (area under the curve [AUC] for NLR=0.64, AUC for PLR=0.59). Recurrent stroke was best predicted by NLR >2.98 (sensitivity, 94.7%; specificity, 60.2%; AUC, 0.84) and PLR >115.9 (sensitivity, 78.9%; specificity, 57.2%; AUC=0.72) (Figure 1).

Based on the ROC analyses, we adopted rounded-off cutoffs of NLR ≥3 and PLR ≥120 for ease of application. We assessed

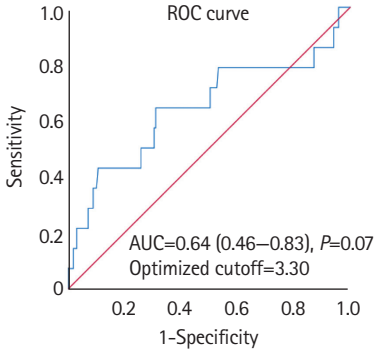
Table 1. Logistic regression analysis of patients with combinations of NLR/PLR and LAVI

Variable	New AF		Recurrent stroke		Composite outcome	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
NLR and PLR for predicting outcomes*						
NLR	1.41 (1.11–1.78)	0.041	2.48 (1.66–3.77)	<0.001	2.50 (1.74–3.61)	<0.001
PLR	1.01 (0.99–1.01)	0.243	1.01 (1.01–1.02)	0.011	1.01 (1.01–1.02)	<0.001
NLR and PLR for predicting recurrent stroke after adjusting for AF and LAVI†						
NLR	-	-	3.47 (1.88–6.39)	<0.001	-	-
PLR	-	-	1.02 (1.02–1.03)	<0.001	-	-
Patients with combinations of NLR and LAVI‡						
Low LAVI and low NLR	1 (Reference)	-	1 (Reference)	-	1 (Reference)	-
Low LAVI and high NLR	3.18 (0.56–18.0)	0.191	18.13 (2.25–145.89)	0.062	9.02 (2.46–33.05)	0.001
High LAVI and low NLR	9.21 (1.41–60.16)	0.022	-	-	6.07 (1.11–33.14)	0.037
High LAVI and high NLR	12.64 (2.26–70.65)	0.004	49.71 (5.77–428.55)	<0.001	40.93 (9.78–171.20)	<0.001
Patients with combinations of PLR and LAVI§						
Low LAVI and low PLR	1 (Reference)	-	1 (Reference)	-	1 (Reference)	-
Low LAVI and high PLR	1.78 (0.32–10.06)	0.512	4.30 (0.90–20.67)	0.068	3.20 (0.99–10.34)	0.052
High LAVI and low PLR	5.08 (0.66–39.37)	0.121	5.08 (0.66–39.37)	0.123	5.82 (1.26–26.77)	0.024
High LAVI and high PLR	11.00 (2.05–59.20)	0.005	11.00 (2.04–59.20)	0.005	16.00 (4.41–58.05)	<0.001

NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; LAVI, left atrial volume index; AF, atrial fibrillation; OR, odds ratio; CI, confidence interval.
*Adjusted for age, sex, hypertension, statin and antiplatelet use, and diabetes mellitus; †Adjusted for age, sex, hypertension, statin use, antiplatelet use, diabetes mellitus, high LAVI, and AF; ‡High NLR defined as ≥3, high LAVI defined as ≥35; §High PLR, defined as ≥120; high LAVI, defined as ≥35.

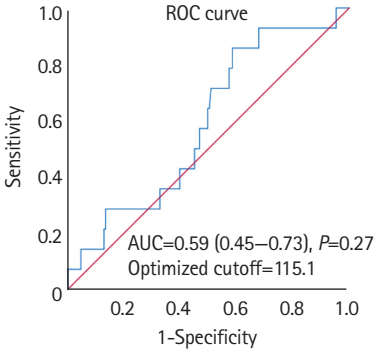
https://doi.org/10.5853/jos.2022.00486

patients with high LAVI and high NLR or PLR for newly diagnosed AF, recurrent stroke, and composite outcomes. A high NLR and LAVI were predictive of new AF (odds ratio [OR], 9.02; 95% confidence interval [CI], 2.46 to 33.05), recurrent stroke



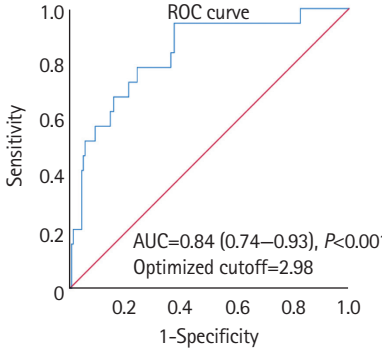
AUC=0.64 (0.46–0.83), *P*=0.07
Optimized cutoff=3.30, sensitivity 0.643, specificity 0.690,
Youden statistic 0.333

A



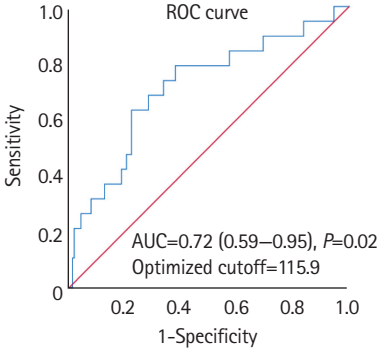
AUC=0.59 (0.45–0.73), *P*=0.27
Optimized cutoff=115.1, sensitivity 0.857, specificity 0.415,
Youden statistic 0.272

B



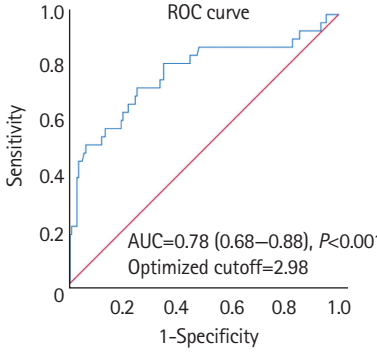
AUC=0.84 (0.74–0.93), *P*<0.001
Optimized cutoff=2.98, sensitivity 0.947, specificity 0.602,
Youden statistic 0.549

C



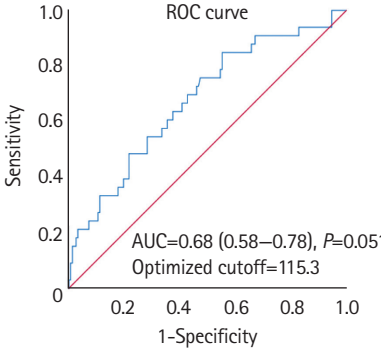
AUC=0.72 (0.59–0.95), *P*=0.021
Optimized cutoff=115.9, sensitivity 0.789, specificity 0.572,
Youden statistic 0.361

D



AUC=0.78 (0.68–0.88), *P*<0.001
Optimized cutoff=2.98, sensitivity 0.818, specificity 0.651,
Youden statistic 0.469

E



AUC=0.68 (0.58–0.78), *P*=0.051
Optimized cutoff=115.3, Sensitivity 0.818, specificity 0.447,
Youden statistic 0.265

F

Figure 1. Receiver operating characteristic (ROC) curves for neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) for atrial fibrillation (AF), recurrent stroke and composite events. (A) NLR and AF, (B) PLR and AF, (C) NLR and recurrent stroke, (D) PLR and recurrent stroke, (E) NLR and combined events, and (F) PLR and combined events. AUC, area under the curve.

(OR, 6.07; 95% CI, 1.11 to 33.14), and the composite endpoint (OR, 40.93; 95% CI, 9.78 to 171.20). A similar finding was observed in patients with high PLR and LAVI. An elevated NLR or PLR with a low LAVI was not significantly associated with new AF or recurrent stroke (Table 1).

This study had several limitations. First, less than half of the patients received an ILR. ILR implantation is affected by financial considerations, since it requires co-payment. Second, none of the patients in this cohort underwent a saline study or transesophageal echocardiography. In our hospital, these tests are performed only if a significant patent foramen ovale is being considered based on TTE.

In conclusion, NLR and PLR were associated with recurrent stroke in patients with ESUS, even after adjustment for comorbidities, AF, and LAVI. The results suggest two different phenotypes of ESUS—one with a strong relationship with atrial cardiomyopathy and AF, and another associated with an inflammatory pathway, atherosclerosis, and systemic disease. Further studies are required to further elucidate these phenotypes and identify more effective and targeted treatments.

Supplementary materials

Supplementary materials related to this article can be found online at https://doi.org/10.5853/jos.2022.00486.

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Received: February 4, 2022
Revised: February 19, 2022
Accepted: February 23, 2022

The authors have no financial conflicts of interest.

Supplementary Table 1. Characteristics of patients with ESUS

Variable	Total (n=185)	Newly-diagnosed AF			Recurrent stroke			Composite outcome (new AF or recurrent stroke)		
		AF (n=14)	OR (95% CI)	*P*	Stroke (n=19)	OR (95% CI)	*P*	Composite (n=33)	OR (95% CI)	*P*
Age (yr)	63.0±12.3	65.3±11.9	2.53 (−4.23 to 9.30)	0.461	61.9±10.3	0.99 (0.95–1.03)	0.681	63.3±10.9	1.00 (0.97–1.03)	0.861
Male sex	128 (70.7)	8 (57.1)	1.92 (0.63–5.82)	0.362	15 (78.9)	0.99 (0.95–1.03)	0.672	23 (69.7)	0.91 (0.40–2.06)	0.821
Ethnicity										
Chinese	128 (69.2)	14 (100)	-	0.082	14 (73.7)	-	0.111	28 (84.8)	-	0.161
Malay	35 (18.9)	0 (0)			4 (21.1)			4 (12.1)		
Indian	14 (7.6)	0 (0)			1 (5.3)			1 (3.0)		
Others	8 (4.3)	0 (0)			0 (0)			0 (0)		
Co-morbidities										
Hypertension	138 (74.6)	12 (85.7)	2.15 (0.46–9.97)	0.521	16 (84.2)	1.92 (0.54–6.92)	0.322	28 (84.8)	2.14 (0.77–5.91)	0.141
Diabetes mellitus	65 (35.1)	5 (35.7)	0.99 (0.32–3.09)	0.992	9 (47.4)	1.77 (0.68–4.60)	0.243	14 (42.4)	1.46 (0.68–3.15)	0.342
Hyperlipidaemia	104 (56.2)	10 (71.4)	2.09 (0.63–6.92)	0.221	12 (63.2)	1.38 (0.52–3.68)	0.524	22 (66.7)	1.71 (0.77–3.77)	0.193
Ischemic heart disease	34 (18.4)	1 (7.1)	0.33 (0.04–2.68)	0.471	7 (36.8)	3.00 (1.08–8.32)	0.031	8 (24.2)	1.55 (0.63–3.82)	0.344
Heart failure	7 (3.8)	0 (0)	-	1.003	3 (15.8)	7.59 (1.56–37.0)	0.011	3 (9.1)	3.70 (0.79–13.39)	0.102
Previous stroke/TIA	32 (17.3)	5 (35.7)	3.01 (0.94–9.71)	0.071	9 (47.4)	5.60 (2.05–15.2)	0.001	14 (42.4)	5.49 (2.35–12.81)	<0.001
Peripheral vascular disease	15 (8.1)	0 (0)	-	0.612	4 (21.1)	3.76 (1.07–13.3)	0.042	4 (12.1)	1.77 (0.53–5.94)	0.361
Laboratory findings										
eGFR (>60 mL/min)	82.4±23.4	73.4±22.9	0.98 (0.96–1.01)	0.151	79.9±26.4	0.99 (0.97–1.01)	0.651	77.0±24.7	0.99 (0.97–1.01)	0.201
HbA1c (%)	6.66±2.02	6.72±2.30	1.01 (0.75–1.37)	0.934	7.26±2.49	1.14 (0.92–1.42)	0.221	7.04±2.39	1.10 (0.92–1.33)	0.292
LDL-C (mmol/L)	3.01±1.15	2.79±0.84	0.83 (0.50–1.38)	0.472	2.87±0.94	0.90 (0.56–1.50)	0.662	2.84±0.88	0.85 (0.59–1.23)	0.391
Hematological parameters										
Total white cell count ($\times10^3$/mm^3)	8.18±2.39	8.58±3.06	1.07 (0.86–1.33)	0.523	9.18±3.03	1.19 (0.99–1.43)	0.061	8.92±3.01	1.16 (0.99–1.16)	0.061
Neutrophil count ($\times10^3$/mm^3)	5.26±2.01	6.08±2.96	1.20 (0.95–1.52)	0.121	6.89±2.66	1.43 (1.16–1.77)	0.001	6.55±2.77	1.42 (1.18–1.72)	0.012
Lymphocyte count ($\times10^3$/mm^3)	2.04±0.83	1.67±0.73	0.47 (0.20–1.10)	0.082	1.48±0.63	0.25 (0.10–0.61)	0.002	1.56±0.67	0.30 (0.15–0.58)	0.011
Hemoglobin (g/dL)	13.52±2.01	14.0±1.50	1.13 (0.85–1.51)	0.401	13.65±2.54	1.03 (0.81–1.31)	0.832	13.77±2.14	1.08 (0.89–1.31)	0.452
Platelet count ($\times10^3$/mm^3)	260.2±81.9	244.4±67.4	1.00 (0.99–1.01)	0.452	259.5±67.2	1.00 (0.99–1.01)	0.971	253.1±66.6	1.00 (0.99–1.01)	0.583
NLR	3.01±1.91	4.48±3.64	1.30 (1.06–1.59)	0.011	5.33±2.88	1.68 (1.28–2.20)	<0.001	4.97±3.20	2.20 (1.59–3.04)	<0.001
PLR	146.8±76.4	173.3±107.6	1.01 (0.99–1.01)	0.182	208.7±108.2	1.01 (1.01–1.02)	0.001	193.7±107.7	1.01 (1.01–1.02)	<0.001
Echocardiographic parameters										
LVEF 30%–50%	25 (1/3.8)	1 (7.1)	1.82 (0.23–14.63)	0.571	3 (15.8)	1.45 (0.39–5.44)	0.581	4 (12.1)	1.03 (0.32–3.26)	0.961
LA volume (mL)	47.4±17.8	62.3±22.0	1.04 (1.01–1.07)	0.002	53.6±15.8	1.02 (0.99–1.05)	0.112	57.3±3.3	1.04 (1.02–1.06)	0.001
LA volume index (mL/m^2)	27.7±10.3	36.6±12.2	1.08 (1.03–1.13)	0.002	32.1±9.2	1.05 (1.01–1.10)	0.031	34.0±10.6	1.07 (1.03–1.10)	<0.001
LA diameter index (mm/m^2)	22.0±4.3	23.7±3.8	1.00 (0.98–1.01)	0.851	22.9±2.7	0.99 (0.98–1.01)	0.812	23.2±3.2	1.00 (0.99–1.01)	0.742
LVEDVi (mL/m^2)	60.5±18.5	58.9±15.0	1.00 (0.96–1.02)	0.763	67.7±31.0	1.02 (0.99–1.04)	0.081	63.9±25.5	1.01 (0.99–1.03)	0.211
LVMi (g/m^2)	99.7±28.7	102.4±27.2	1.01 (0.98–1.02)	0.734	104.9±28.2	1.01 (0.99–1.02)	0.411	103.8±27.4	1.01 (0.99–1.02)	0.382

Values are presented as mean±standard deviation or number (%).
ESUS, embolic stroke of undetermined source; AF, atrial fibrillation; OR, odds ratio; CI, confidence interval; TIA, transient ischemic attack; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; LDL-C, low-density lipoprotein cholesterol; NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; LVEF, left ventricular ejection fraction; LA, left atrium; LVEDVi, left ventricular end-diastolic volume index; LVMi, left ventricular mass index.